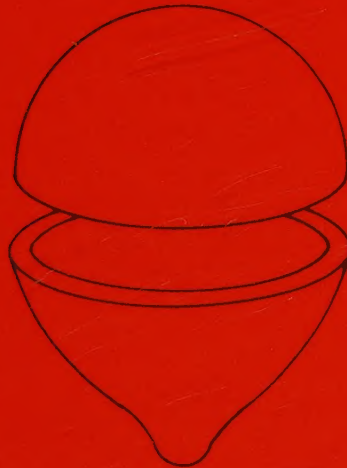


ACTA PHYSIOLOGICA SCANDINAVICA

Supplementum 483

RELATION BETWEEN
MECHANICAL AND MORPHOLOGICAL CHARACTERISTICS
IN URINARY BLADDER SMOOTH MUSCLE

BY
BENGT UVELIUS



LUND 1980

RELATION BETWEEN MECHANICAL AND MORPHOLOGICAL
CHARACTERISTICS IN URINARY BLADDER SMOOTH MUSCLE

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Abstract Mechanical characteristics of smooth muscle from rabbit and guinea-pig urinary bladder were examined in relation to bladder morphology. Muscle cell length (determined by nitric acid maceration or morphometry on fixed tissue) was directly proportional to bladder radius (or stretch of isolated bladder strips) indicating a series coupling of the cells. The number of cross-sectioned cells per mm ² varied between 35000 and 170000 and was found to increase in proportion to bladder radius, indicating that cell volume is not affected by passive length changes. Length-tension relations (L-T relations) were determined for rabbit bladder strips. Active force was 19 N/cm ² at optimum length. The strips could shorten actively to 17 % of this length, when unloaded. During the major portion of the rising part of the L-T curve isometric and isotonic L-T relations were identical. At greater lengths the strips failed to shorten to the same length as when contracting from a shorter starting length. L-T relations for a single guinea-pig bladder cell were calculated. Max active tension at optimum length (400 µm) was 5.5 µN/cell corresponding to 59 N/cm ² pure muscle. Isotonic quick releases were performed on rabbit bladder strips. The immediate series elastic recoil (SE recoil) was followed by a transient phase (75 ms) of rapid shortening, and finally a steady shortening. Max SE recoil was 4 %. SE characteristics were unaffected by mode of stimulation suggesting that it is predominantly passive. V _{max} was 0.37 l/s and 0.26 l/s when strips were activated by AC and K ⁺ -high media, respectively. The rate constant for the transient was lower for K-activation than for AC, indicating that it is due to events associated with the contractile proteins. Hypertrophic bladder muscle had lower max active tension than controls, but the difference disappeared after correction for higher water content in the former. Hypertrophic bladders could not shorten to the same ultimate length, when unloaded, as their controls.			
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Supplementum 483

From the Institute of Physiology and Biophysics

University of Lund

Sweden

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MECHANICAL AND MORPHOLOGICAL CHARACTERISTICS
IN URINARY BLADDER SMOOTH MUSCLE**

BY

BENGT UVELIUS

LUND 1980

This summary contains, apart from previously unpublished data, results from the following papers:

- I UVELIUS, B. 1976. Isometric and isotonic length-tension relations and variations in cell length in longitudinal smooth muscle from rabbit urinary bladder. *Acta Physiol Scand* 97: 1-12.
- II UVELIUS, B. 1977. Influence of muscle length on the force-velocity relation of K^+ -contractures in smooth muscle from rabbit urinary bladder. *Acta Physiol Scand* 101: 270-277.
- III JOHANSSON, B., HELLSTRAND, P. & UVELIUS, B. 1978. Responses of smooth muscle to quick load change studied at high time resolution. *Blood Vessels* 15: 65-82.
- IV UVELIUS, B. 1979. Shortening velocity, active force and homogeneity of contraction during electrically evoked twitches in smooth muscle from rabbit urinary bladder. *Acta Physiol Scand* 106: 481-486.
- V UVELIUS, B. & Hellstrand, P. 1980. Effects of phasic and tonic activation on contraction dynamics in smooth muscle. *Acta Physiol Scand*, in press.
- VI UVELIUS, B. & GABELLA, G. 1980. Relation between cell length and force production in urinary bladder smooth muscle. Submitted to *Acta Physiol Scand*.

The papers are referred to in the text by their Roman numerals.



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1. GENERAL INTRODUCTION

Due to intense research over many years, our present knowledge is large regarding morphology, energetics, mechanical properties etc of skeletal muscle. The sliding filament concept of contraction was established some 25 years ago (for review see e.g. Huxley 1971). Research on smooth muscle mechanics has been hampered for several reasons. The experimental procedures have been difficult to standardize and it has been difficult to obtain reproducible results. Katz showed, however, already in 1939 that Hill's (1939) equation is applicable also to smooth muscle by experiments on tortoise retractor penis muscle. In more recent years Katz' results have been confirmed and extended for a variety of mammalian smooth muscles (e.g. Åberg & Axelsson 1965, Hardung & Laszt 1966, Stephens, Kroeger & Mehta 1969, Mashima & Handa 1969, Gordon & Siegmán 1971, Hellstrand & Johansson 1975). From these studies it can be seen that smooth muscle in general has lower maximal shortening velocity ($V_{\max}$) than skeletal muscle and that isometric tension for most preparations is of the same magnitude as for skeletal muscle. There is great variation between results obtained from different smooth muscles regarding $V_{\max}$. Whereas $V_{\max}$ was only 0.03 lengths/s for rabbit taenia coli at 22°C (Mashima & Handa 1969) it was 0.74 l/s for rat portal vein at 37°C (Hellstrand & Johansson 1975). Part of this difference is probably due to different methods (afterloaded isotonic contractions or quick release) and temperature but most probably different smooth muscles possess individual characteristics.

The mechanical behaviour of a smooth muscle depends (among other things) on the characteristics of the contractile proteins and on the relation between neighbouring cells since smooth muscle preparations are multicellular. During recent years it has been shown (see Somlyo & Somlyo 1975) that smooth muscle contains thick and thin myofilaments although not as regularly arranged as in skeletal muscle. The intercellular relations are complicated. In taenia coli from guinea pig (Gabella 1976c) the cells are anchored to each other and to collagen fibrils along the cell surface and at the end of the cells complicated interdigitations are observed.

If mechanical properties determined for smooth muscle preparations are to be considered relevant for the individual cell, one has to know how the cells behave in comparison to the whole preparation, e.g. how the lengths of the cells change in proportion to a length perturbation of the whole

preparation. The most straight-forward way to avoid this difficulty is to study the behaviour of isolated individual cells. It is possible to obtain viable single cells from toad stomach digested with trypsin and collagenase (see review by Fay, Cooke & Canaday 1976). Such cells can be mounted for recording of contractile force during activation for instance by electrical stimulation. It is not certain however, that the mechanical output of an isolated cell represents the behaviour of a cell in situ, anchored as it is, not only at its ends but also laterally to neighbouring cells and to collagen fibrils present in the space between the cells.

Another way to obtain information regarding the mechanics of the individual cells is to perform the relevant experiments on multicellular preparations in which the behaviour of the individual cells in relation to the preparation at a whole is known. With this approach and the above mentioned background the present study was performed.

Scope of present investigation

The preparations used are rabbit and guinea pig urinary bladder. Some experiments are performed on whole bladders in vivo, and others on strips of longitudinal bladder muscle in vitro. The reason for the choice of this preparation (which has not been used much previously for mechanical studies) was mainly that the length of the isolated preparations could be increased considerably without any marked increase in passive tension. The bladder as a densely innervated organ with only minor spontaneous activity is also easy to control and stimulate. Some comparison is made with results on rat portal vein which has considerable passive tension and a phasic spontaneous activity. This is done with the notion that conclusions regarding smooth muscle in general can be drawn with greater certainty if comparative results are obtained from different smooth muscle types.

The results of the morphological and morphometrical studies of the bladder preparations are accounted for in chapter 2. The effects of bladder distension on cell length and cell diameter in whole bladders and of length changes in isolated strips are shown.

Chapter 3 deals with the mechanics of bladder muscle. Length-tension relations for single smooth muscle cells are calculated from volume-active-pressure relations in vivo. An analysis is given of the applicability of the isotonic quick release method (Jewell & Wilkie 1960) for the study of

mechanics of isolated strips. The behaviour of the muscle strips during active shortening and the effect on this of different modes of stimulation are investigated. A study of the capability of the bladders to adapt to an introduced residual volume is given in chapter 4. Chapter 5 contains a general discussion and a summary.

2. MORPHOLOGICAL ASPECTS

2:1 Introduction

The mammalian smooth muscle cell in general is thin and slender. The dimensions vary depending on the organ and on the functional state. (see rev. by Burnstock 1970). The mid-cell diameter ranges from 1.5 to 6 μm and the length from 30 to 450 μm . Earlier ultrastructural studies of urinary bladder smooth muscle cells (Nagasawa & Suzuki 1967, Larsen 1977) show that bladder cells conform to this general description. The length and diameter of rabbit bladder cells were estimated to be about 200 μm and 5 μm for relaxed and 100 μm and 9 μm for contracted cells (Larsen 1977). Both thick and thin myofilaments were observed. Available data is, however, not sufficient for a proper understanding of cellular events in relation to the behaviour of the bladder as a whole. The aim of the studies reviewed in this chapter is to provide such quantitative information that will be necessary for the interpretation of the results from the mechanical experiments in chapter 3. From a description of the remodelling of the muscle wall when the bladder is inflated (2:3, 2:4), attention will be focused on the behaviour of the single muscle cell in situ. Cell length (2:5) and number of cells per mm^2 (2:6) will be described in relation to bladder radius. In 2:7 an attempt will be made to determine how cell volume is affected by bladder distension. Finally (2:8) morphological characteristics are given for the isolated bladder strips used in chapter 3.

2:2 Outline of methods

Adult guinea-pigs (body weight 300-500 g) and rabbits (body weight 2500-3500 g) were used. The general processing is outlined below. There will be divergences from this scheme in section 2:8 but they will be accounted for there.

The animal was killed and the bladder was excised, emptied and transferred to oxygenated Krebs solution (paper VI). The urethra was then cannulated and the bladder slowly filled to a desired volume either through a gravity fall or by stepwise injections with a syringe connected to the cannula. Filling could take up to 30 min for the highest volumes. The bladder was then transferred to a glutaraldehyde solution at room temperature. After about 10 min the bladder was cut open, divided into identifiable parts which were transferred to fresh fixative. Some bladders were fixed with their content of urine immediately after ligation of the urethra and removal

from the animal. After 10 min in fixative the bladder was slit open and the content volume was measured. The bladders were then treated as described in the foregoing. The preservation of fine structures was not good in these bladders, perhaps due to leakage of urine into the bladder wall during fixation.

After the fixation period (2-6 h) the specimens were immersed for 1-2 h in 2 % osmium tetroxide and then transferred for 30 min to a 1 % aqueous solution of uranyl acetate. After dehydration with ethanol and expoxypropane, the specimens were infiltrated with Araldite. After polymerization suitable blocks were cut with glass knives. Sections 1.5 - 3.5 μm thick for light microscopy were collected on microscope slides, examined and photographed unstained in a phase contrast microscope. Sections for electron microscopy were collected on grids, stained with lead citrate and examined in a Philips 300 electron microscope. All histological observations were carried out on detrusor muscle from the mid-ventral part of the bladder.

When cross sections should be made on muscle bundles in a block, the exact direction of the bundle was visualized under a dissection microscope and appropriate cuts could be made. Bladders with small content volumes were not translucent enough to make it possible to see the bundles. Under such circumstances it was necessary to make serial sections for phase contrast microscopy to ensure that a bundle was at right angle to the direction of the cuts. Generally a bundle was followed over at least 1 mm.

Bladder radius will be expressed as bladder outer radius. This is calculated from the "bladder outer volume" which is obtained by the addition of the bladder wall volume to the content volume. The reason for doing this is that the muscle layer is much closer to the outer than to the inner surface due to a submucosa of considerable thickness. In other experiments the weights of guinea pig bladders were found to be around 0.3 g, and therefore 0.3 ml is added to all content volumes in order to obtain outer volumes. Mean rabbit bladder weight was 1.8 g and 1.8 ml is added to the content volumes in rabbit bladders in order to obtain outer volumes.

Bladder radius and circumference are calculated from bladder volume assuming spherical form. The guinea-pig bladder is in fact almost spherical at all volumes studied (0 - 6.7 ml) whereas the rabbit bladder is slightly elongated.

Quantitative results are given as mean $\pm$ standard error (SE). Number of observations are given in parenthesis.

2:3 Variation in the muscle layer thickness with changes in bladder volume

If distension of the bladder portion that is used in this study (the midventral portion) is to be calculated from the bladder volume, the assumption has to be made that all parts of the bladder wall are stretched by the same relative amount in response to increases in bladder volume. It can be expected a priori that one part of the bladder wall, namely the trigonum, is more resistant to stretch than the rest, but the trigonum constitutes only a small portion of the bladder wall. In this section the applicability of the assumption that all parts of the guinea pig bladder are stretched to the same extent will be considered.

Araldite-embedded midventral portions from 6 guinea pig bladders were used. The bladders were fixed with content volumes between 0.25 ml and 6.7 ml. The muscle wall thickness was measured with a calibrated eye piece in the phase-contrast microscope. 15 determinations were performed for each of the six bladders. The results are shown in Fig. 1. It is seen that the muscle wall thickness decreases when the bladder outer radius is increased. At a bladder outer radius of 0.51 cm (corresponding to a content volume of 0.25 ml and an "outer volume" of 0.55 ml)

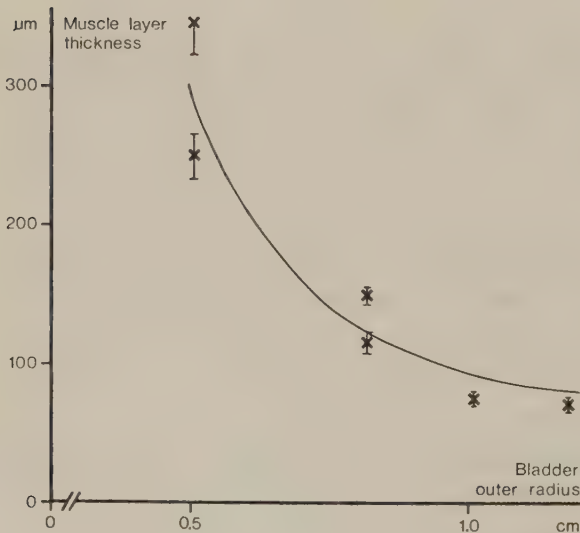


Fig. 1 Thickness of total muscle layer of 6 guinea-pig bladders filled to different volumes. Bladder outer radius represents radius of the content, assuming spherical shape, plus total bladder wall thickness. Full line indicates expected behaviour calculated from the volume of the muscle layer at the lowest bladder volume, assuming that all parts of the bladder are equally distensible. Each point represents mean of 15 thickness determinations.

the mean muscle layer thickness of the 2 bladders is 300 μm . This gives a calculated total volume of the muscle layer of 92 mm^3 . If all parts of the bladder wall are stretched to the same extent when the bladder is inflated and if the volume of the muscle layer can be considered independent of content volume, muscle layer thickness should vary in accordance with the full line in Fig. 1. The thickness measurements agree well with what is to be expected if the above mentioned assumptions are relevant.

The results in this section thus show that a certain relative increase in guinea pig bladder radius results in the same relative length change in the particular segment of the bladder wall used in the experiments (the mid-ventral portion).

2:4 Rearrangement of muscle bundles with increasing bladder volume

The muscle wall of the bladder is considered to consist of three ill-defined layers, an outer and an inner longitudinal and an intermediate circular layer (see e.g. Ham 1969). The circular layer is usually the thickest. This description seems to be valid also for the bladders used in this study. Each layer seems to consist of muscle bundles separated from each other by connective tissue. The direction of the bundles in each layer is not uniform. Bundles with a direction almost at right angle to the others are often found in the different layers. The expressions circular and longitudinal layer therefore express only the general direction of the muscle bundles within each layer.

The thickness of the muscle layer as a whole decreases when the bladder is inflated as shown in section 2:3. In the phase-contrast microscope it can be seen that cross-sectioned bundles in sections from distended bladders seem to be somewhat flattened when compared to bundles from bladders with a low content volume. This flattening is however not prominent. The profiles of individual cells in cross-sectioned bundles are, by judging from the electron micrographs, somewhat flattened in the distended bladders but much less so than would be expected if the bundles were stretched laterally in proportion to the increase in bladder diameter.

The phase-contrast micrographs (VI) suggest that the muscle bundles spread laterally when the bladder is inflated, i.e. that the radial number of bundles decreases with increasing bladder volume. This was a general

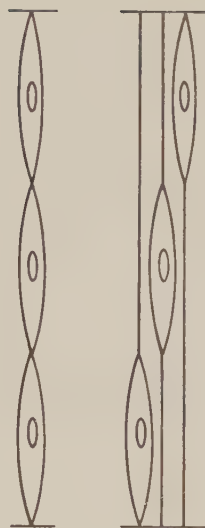
impression for the 6 guinea-pig and 8 rabbit bladders studied. If this is correct the number of muscle cells across the thickness of the wall would decrease when the bladder is inflated. Therefore the number of muscle cells across the full thickness of the muscle coat was determined by counting how many muscle cells were intersected by radial lines drawn orthogonally through the wall. These determinations were performed either on phase-contrast micrographs or directly in the microscope. The results show that the radial number of cells decreases with increasing bladder radius for all volumes studied (VI). This is to be expected if, as suggested above, the muscle bundles spread laterally when the bladder is inflated.

There are two additional conclusions to be drawn from the results presented above. 1. it is difficult if not impossible to dissect out strips for in vitro experiments where all cells have the same direction. Even in strips from one muscle layer bundles with a considerable oblique direction in relation to the long axis of the preparation will be included. This will be of relevance for the in vitro determination of force per unit cross sectional area (see chapter 3). 2. as the muscle bundles in the wall tend to spread tangentially more than being flattered when the bladder is inflated, length variations for isolated strips (where the bundles are not flattened) will be a good model of the individual bundle in situ. If the bundles had become considerably flattened in situ the geometrical arrangements of the cells within a bundle might have changed with bladder volume. Then it would have been much more difficult to transfer results from strips in vitro to the whole bladder.

2:5 Muscle cell length in relation to bladder distension

If results from mechanical experiments on whole bladders or bladder strips are to be carried over to individual cells in the preparation, it is essential to know how the cells are coupled to each other. Fig. 2 shows schematically 2 extreme arrangements, i.e. series (SC) and parallel coupling (PC). Intermediates between these are also possible. If the individual cells in the figure produce the same force, then the force output of the model with 3 parallel coupled cells would be 3 times greater, whereas the SC preparation gives just the same force as one cell. If the length of the preparations is doubled by passive stretch, then the cell length in the SC model is also doubled, whereas cell length for the PC model increases to a much greater extent. These are drastic examples showing the necessity

Fig. 2 Alternative arrangements of muscle cells within a tissue. The cells coupled in parallel (right) produce per unit cross-sectional area 3 times the force attained by the preparation with the cells coupled in series (left). The non-cellular force-transmitting structures in the right panel are assumed to be rigid. Modified from Murphy (1976).



of knowing the cellular coupling if cellular behaviour in intact preparations is to be calculated.

If cell lengths are determined for preparations subjected to different degrees of stretch the cellular coupling can be determined (see Murphy, Driska & Cohen 1977). Should the cells be coupled in series a direct proportionality between changes in preparation length and cell length is to be expected. Should they be parallel coupled the relative length change of the cells is greater than for the preparation as a whole, the magnitude being dependent on the degree of parallel coupling.

In order to study the dimensions of individual cells, Anopolsky (1928) obtained suspensions of isolated smooth muscle cells from pig fallopian tube by maceration of unfixed tissue pieces in 30 % nitric acid. Cooke & Fay (1972) macerated glutaraldehyde fixed strips of guinea-pig taenia coli in 25 % KOH. The method used in paper I is a modification of the method devised by Anopolsky. Rabbit bladders were filled to desired content volumes and placed for 1 h in Ca-free Krebs solution containing 1.0 mM EGTA in order to chelate Ca^{2+} and thereby ensure that the cells were in a relaxed state. The bladders were fixed in an isotonic, buffered formaldehyde fixative. After 24 h in the fixative longitudinal strips of the bladders

were dissected out. These strips were then placed in increasing concentrations of nitric acid, 1/2 h in each, starting with 0.6 % (roughly isosmolar). The strips were finally placed in 30 % HNO_3 for 4 days. The rationale for the stepwise increase in concentration was to avoid possible effects on the cells such as shrinkage induced by drastic changes in osmolality of the bathing medium. When the macerated strips were gently stirred after 4 days in nitric acid, a suspension of isolated cells and cell conglomerates was obtained. A drop of this suspension was placed on a slide and a cover slide was put on. Isolated individual cells were photographed and the lengths were measured on the prints. Only isolated, intact, symmetrically formed cells with well tapered ends were considered.

In order to ascertain that the muscle cells were not activated during fixation and that they did not shrink when they were placed in the nitric acid solutions, tension of some longitudinal strips was measured continuously during these procedures. No tension developed which strongly suggests that the cells were fixed in a relaxed state.

Filled circles in Fig. 3A show the results of the cell length determinations. It is clearly seen that an increase in bladder radius is accompanied by a closely similar relative length change of the individual cells. The results agree qualitatively with those of Cooke & Fay (1972) on guinea-pig taenia coli although they have studied cells from only two preparation lengths. The results are also well in accordance with those on pig carotid artery reported by Murphy, Driska & Cohen (1977), Driska & Murphy (1978), and Driska, Damon & Murphy (1978).

There is some uncertainty regarding the reliability of the above study since the length of the cells might possibly change during the maceration of the strips despite that no tension development was observed. Such tension measurements can probably not account for all possible changes in cell length during the maceration, especially not during the later phase when the cells begin to loosen from each other. It was also felt that length changes, if they occurred, might be of different importance at different degrees of stretch.

The most reliable data regarding cell length are most probably obtained from serial electron microscope sampling. Such studies have been done (e.g. Bennet & Rogers 1967, Merrillees 1968), but the approach has not been

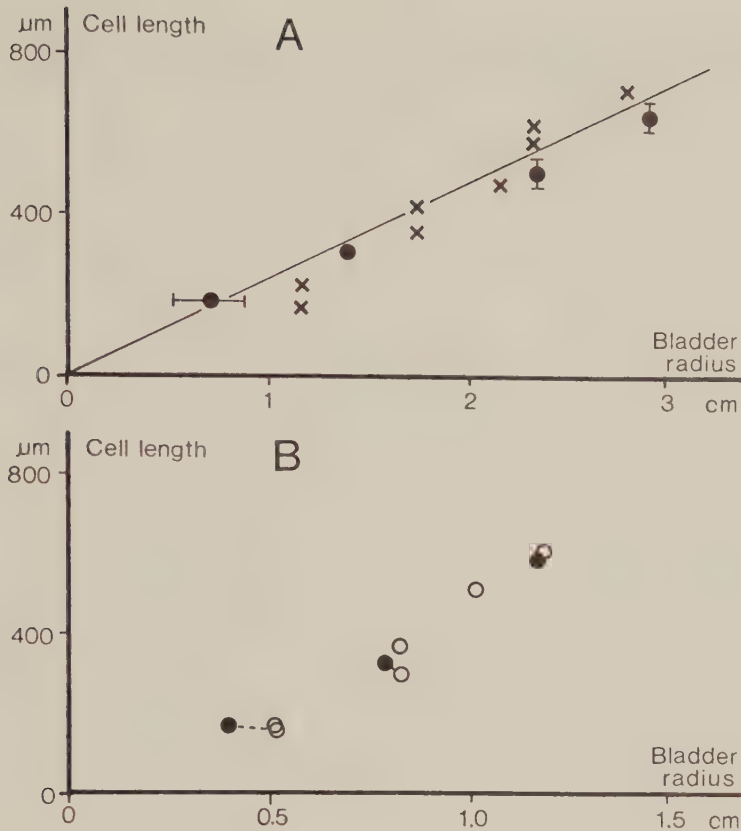


Fig. 3 Muscle cell length vs bladder outer radius. A. Rabbit. Filled circles indicate determinations on nitric acid macerated bladders. 3 bladders at each volume. Vertical bars indicate SE ($n = 53 - 79$), horizontal bars wall thickness. Crosses show morphometrically determined lengths of longitudinal muscle cells. The line shows the expected behaviour if the cells were coupled in series. B. Guinea-pig. Open circles: longitudinal muscle, filled circles: circular muscle. For longitudinal muscle radius is given as outer radius, for circular muscle as inner radius. Points connected by broken lines indicate results from same bladder. From (I) and (VI)

applied to the study of different degrees of stretch. It would probably be too laborious to obtain a reasonable number of length determinations in such an investigation.

Halpern, Mulvany & Warshaw (1978) have used Nomarski interference contrast optics to measure distance between intracellular structures of the same muscle cell in segments of small rat mesenteric arteries in vitro. Increases

in internal circumference by up to 50 % in inactive vessels resulted in a nearly proportional increase in spacing between the intracellular markers. The results regarding the part of the muscle cell between the markers thus correspond well with those described above. These authors have, however, no results regarding the behaviour of the tapering ends of the cells.

Gabella (1976a) has devised a morphometric method to determine muscle cell length:

$$\text{mean cell length} = \frac{\text{mean nucleus length} \times 100}{\% \text{ of cross-sectioned cell profiles containing nuclei}}$$

The advantage of this method is that the cells are left in situ thus minimizing the risk that length changes could occur. This method was applied to 6 guinea-pig and 8 rabbit bladders. Cross sections for EM were cut from identified longitudinal muscle bundles and the number of cross sectioned cells were counted as were the number of cells containing nuclear profiles. Then the tissue blocks were re-embedded in Araldite and tangential sections for phase-contrast microscopy were cut from the bundles chosen above. Lengths of nuclei with identifiable nuclear poles were measured with a calibrated eye piece. With guinea pig bladder the same method was used also for determining cell length in the circular muscle layer. The number of cross-sectioned cell profiles counted in each determination ranged between 448 and 2252 and the number of nuclei between 26 and 115. The majority of the cell profiles are smooth indicating that these cells are fixed in a relaxed state. A fraction of the cells have, however, rough surfaces and protrusions which indicates that these fibres were fixed in an activated state (regarding such protrusions see e.g. Gabella 1976b).

The calculated cell length determinations are shown for rabbit bladder cells in Fig. 3A and they are similar to those obtained by nitric acid maceration. It thus appears that the less tedious nitric acid method gives data as reliable as those obtained by the method of Gabella. The full line in Fig.3A shows the expected cell length variations in relation to rabbit bladder radius if the cells were coupled in series. The experimental data fit closely to this line. According to Fig. 3B a certain relative increase in guinea pig bladder radius is accompanied by a slightly larger relative length increase of the muscle cells. This suggests that there may be some degree of parallel coupling in this preparation. The deviation from the

series coupling behaviour is however so small that the cells in the guinea pig bladder can be considered, in general, to be coupled in series.

It is not known if smooth muscle cells shorten when activated if the preparation as a whole is kept at constant length. The bladders used for nitric acid maceration were pretreated with EGTA and no comparison was made with bladders fixed while activated. The morphometric determinations described above were done on bladders that were not depleted with Ca^{2+} , the fixative itself had 0.5 mM Ca^{2+} added. The electron-micrographs also showed that some cells were fixed in an activated condition. Despite this, cell length was almost the same as for the macerated, inactive bladders. The cell length determinations by Driska & Murphy (1978) on pig carotid artery were done on K^{+} -activated strips alone. Mulvany and Warshaw (1979) were unable to measure distance between intracellular markers for activated mesenteric arteries in vitro due to movements within the preparation. They found however that nucleus length was, on average, little changed. This suggested, according to these authors, that cell length was on average not substantially affected by activation. Fig. 4 shows nucleus length vs bladder radius for rabbit bladder. It is seen that bladder radius, and thus cell length, can vary considerably without much change in length of the nucleus. At least for this preparation nucleus length does not seem to correlate well with cell length.

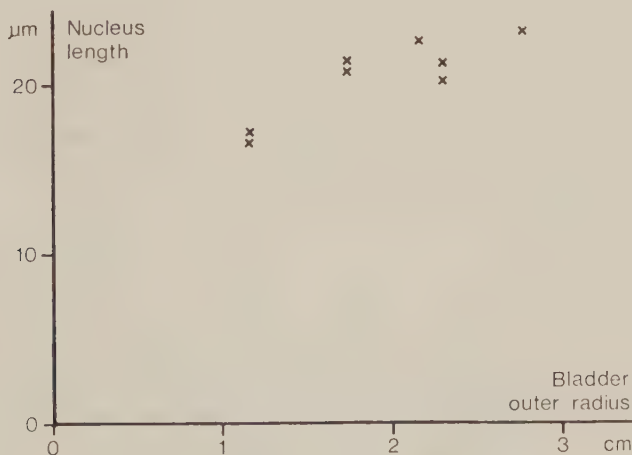


Fig. 4 Length of nuclei in longitudinal muscle from rabbit bladder. Each point represents mean value from 35 nuclei in one bladder. 8 bladders were used. Only nuclei with well identified clear zones around their nuclear poles were considered.

While no conclusive results exist regarding lengths of activated cells vs passive, it my impression that such length changes, if they occur, are small. This statement based on the fact that no significant length difference was found between passive, macerated bladder muscle cells and morphometrically determined cell lengths of bladders fixed in the presence of Ca^{2+} where some cells showed signs of being fixed while active (Fig. 3A).

2:6 Number of cells/unit area: Dependence on bladder volume

It was shown in section 2:5 that cell length increased in a bladder muscle preparation when the length of the preparation is increased. The number of cells per unit area ("cell packing density") in cross-sectioned muscle bundles would then be expected to rise with increasing stretch of the preparation.

Results regarding cell packing density of guinea pig taenia coli strips fixed at different lengths have been reported (Gabella 1976b). In one experiment a taenia strip was fixed at resting length, whereas a contiguous strip was allowed to shorten actively down to 24 % of resting length. Packing densities were $94000 \text{ cells} \times \text{mm}^{-2}$, and $18000 \text{ cells} \times \text{mm}^{-2}$, respectively. In a similar experiment the packing density was $105000 \times \text{mm}^{-2}$ in the stretched strip and $37000 \times \text{mm}^{-2}$ in the strip fixed when shortened to 40 % of initial length.

In the present study on guinea pig urinary bladder cross-sectioned muscle bundles were marked out on EM-plates (VI), the cell numbers were counted, and the contours of the bundles were copied onto transparent film sheets. These contours were then cut out and weighed. By knowing the weight of a calibration area the cross sectional area of the bundles could be calculated. Fig. 5 gives the results. Each point is based on 184-405 cells. For the whole radius interval studied, cell packing density increases when the bladder is inflated. The straight line, obtained by linear regression analysis, fits well ($r = 0.91$) with the experimental data.

Only four rabbit bladders were studied. In the two preparations fixed with a content volume of 5 ml packing density in the longitudinal muscle was 86000 and 64000 per mm^2 , respectively. From the mean values of these ($75000 \times \text{mm}^{-2}$) the figure at 20 ml would be expected to be $119000 \times \text{mm}^{-2}$ ($4^{1/3} \times 75000$). The actual data for two bladders fixed at this volume were 117000 and 141000 (mean 129000), which is close to the expected value.

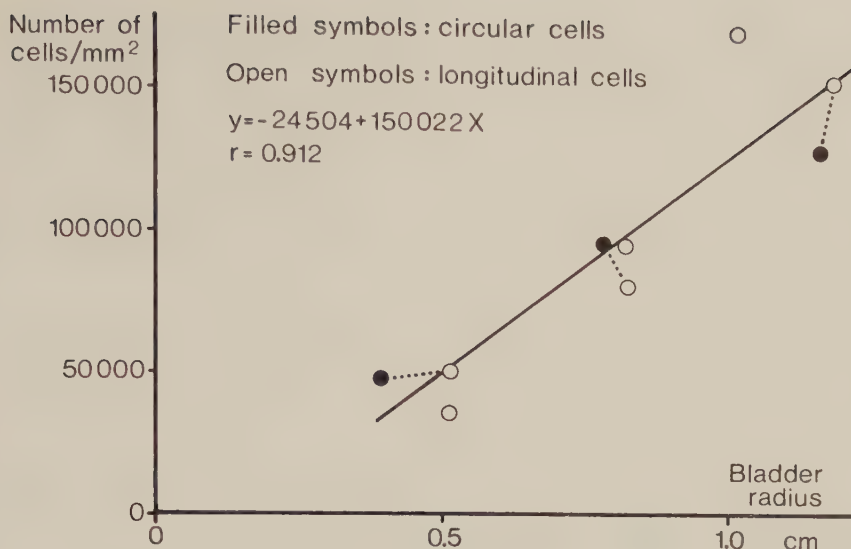


Fig. 5 Number of cells per mm² cross-sectional area vs bladder outer radius. Broken lines connect values from same bladder. The best fitting line according to linear regression analysis is given. From VI.

The results in this section, as well as those in section 2:5 (increase in cell length with bladder radius) suggest that no slippage between cells occur even at great distension of the bladder wall. The importance of this finding will be discussed in chapter 3 with regard to possible explanations for the broad and flat length-active tension curve that will be shown to exist for this type of muscle.

2:7 Effects of passive stretch on cell volume

According to Fay & Delise (1973) smooth muscle cells from toad stomach decrease in volume when they shorten actively. As the cells shortened to 30 % of their resting length the calculated cell volume decreased to 80 % of the initial value.

The results from paper VI reported under 2:5 and 2:6 permit us to speculate on the effect of bladder distension on cell volume. Fig 6 is a composite of the data in Figs 3B and 5. The relation between cell length and cell packing density is well approximated by a straight line in that a doubling of cell length is accompanied by a doubling of the cell packing density. This would indicate that cell volume does not change with length if the

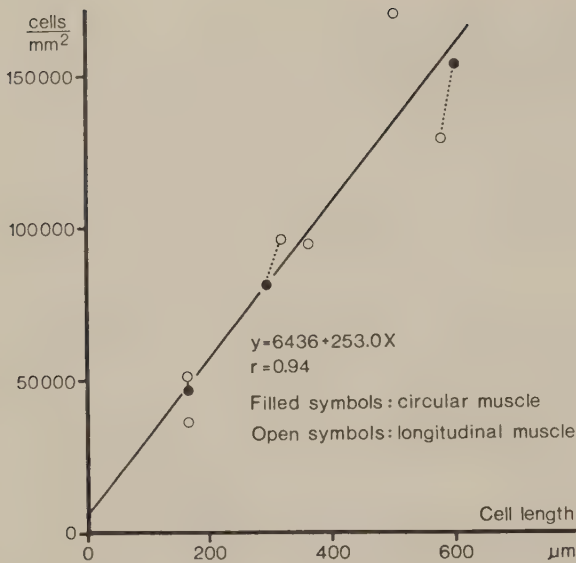


Fig. 6 Number of cells per mm² cross-sectional area vs cell length. The figure is composed of results from Fig. 3B and Fig. 5. Broken lines connect results from same bladder. Best fitting line obtained by linear regression analysis is shown.

amount of intercellular space was constant. The problem of the constancy of this space with changes in muscle length has not yet been investigated by use of specific tracers such as Co-EDTA. However, microscope observations by Gabella (personal communication 1980) indicated that the size of the extracellular space is length-independent in guinea-pig taenia coli.

While the results of my own studies may not be conclusive on this point, they do suggest that cell volume does not change much when cell length is varied. The discrepancy between these findings and the results of Fay & Delise (1973) might be due to the fact that they studied active shortening while the changes in bladder cell length were passive.

2:8 General morphological description of isolated strips used for mechanical experiments

The strips that were used for mechanical experiments in chapter 3 were blotted and weighed afterwards in order to determine their cross-sectional area. This made them unsuitable for histological study. For the latter purpose strips composed mainly of longitudinal muscle were dissected out from rabbit bladders filled with 10 ml Krebs solutions. Either the bladders were fixed before dissection, or the strips were fixed at their in situ length.

In one series of 6 strips the preparations were fixed in glutaraldehyde, embedded in plastic and cross-sectioned for phase-contrast microscopy. From photomicrographs the relative area of smooth muscle was measured to be $67 \pm 3 \%$.

In another series of 6 strips the tissues were fixed in Bouin's solution and then paraffin-embedded. Cross sections and longitudinal sections, 7 μm thick, were cut and stained with resorcin-fuchsin and picric acid - thiazin red for differentiation between smooth muscle, elastic fibrils, and collagen (Romeis 1968). The muscle bundles were found to be surrounded by loose connective tissue containing considerable amount of collagen. The relative cross-sectional area consisting of muscle was the same as for the series described above. Only a very limited number of elastic fibrils was seen among the muscle bundles. A high amount of elastic fibrils was found around intramural vessels and in concomitantly embedded rat portal veins.

On electron-micrographs of both rabbit and guinea pig bladder, collagen fibrils are seen surrounding the individual muscle cells. No further study regarding their relationship to the muscle cells was done (regarding cell-collagen relationship see Gabella 1977).

3. MECHANICAL PROPERTIES

3:1 Introduction

Active force produced by a skeletal muscle increases with increasing extension of the muscle, until a maximum is reached. Further stretch leads to a progressively decreasing active force. The mechanism behind this length-active tension relation was long obscure but certain features of it has now been adequately explained (Gordon et al 1966). The decrease in force seen when the muscle is stretched beyond the optimal length coincides with the decreasing overlap between the thick and thin myofilaments. Thus fewer cross-bridges can participate in force production. The decrease in force at lengths shorter than optimal has been more difficult to explain. An increasing internal load could be of importance in this part of the length-tension diagram (Gordon et al 1966) but evidence has been presented that the electromechanical coupling, especially in the central parts of the muscle cell becomes less efficient at shorter lengths (Rüdel & Taylor 1971).

Smooth muscle has a length-tension relation that, in principle, has the same features as that of skeletal muscle (see e.g. Speden 1960, Csapo 1962, Stephens, Kroeger & Mehta 1969, Halpern, Mulvany & Warshaw 1978, paper I). An important difference is, however, that smooth muscle preparations can develop tension at much shorter length, relative to the optimal, than skeletal muscle. Section 3:3 will deal with the length-tension relation of rabbit bladder strips and possible explanations regarding the shape of it. Section 3:4 contains an attempt to determine the length-active tension relation of single guinea-pig bladder cells.

The relationship between load and shortening velocity is an important characteristic of the contractile machinery. Hill (1938), in a classical series of experiments, showed that shortening velocity (V) can be described by a hyperbolic function of the force (P):

$$V(P + a) = b(P_0 - P) \qquad \text{eqn. 1}$$

This equation represents a rectangular hyperbola with asymptotes at $V = -\frac{b}{a}$ and $P = -a$, and a P axis intercept at $P = P_0$, the isometric force. Until recently it was thought that the constant a reflected directly the energy liberated during shortening. Later investigations have cast doubts on this direct relation between thermal and mechanical events (see e.g. Simmons &

Jewell 1974). The force-velocity relation is, however, still of great importance as a means of characterizing properties of the contractile system.

The muscle model suggested by Hill (1938) separates a contractile component and an undamped series elastic element. During an isometric contraction the contractile element was thought to shorten, thereby stretching the series elastic element. A sudden change in the load of an isotonic shortening muscle was considered to cause an immediate adjustment of the length of the series elastic element and of the shortening velocity of the contractile component according to its force-velocity relation. Thus with this model no transient behaviour is to be expected. Experiments with this theoretical background has been performed on smooth muscle which has been found to behave qualitatively as other muscle types. $V_{\max}$ is however much lower than in skeletal muscle (see e.g. Stephens, Kroeger & Mehta 1969, Gordon & Siegman 1971, Herlihy & Murphy 1974, Hellstrand & Johansson 1975).

During recent years the two-component model by Hill has been challenged mainly as a result of new studies on skeletal muscle. A major part of the elasticity of the active muscle in well controlled studies is now considered not to be directly in series with the contractile machinery, but to be associated with the cross-bridges themselves (Huxley & Simmons 1971, Ford, Huxley & Simmons 1977). Moreover the shortening velocity does not change instantaneously to a new steady level after a load change. The adjustment of the kinetics of the crossbridges takes a certain time (Civan & Podolsky 1966). The same is the case with regard to force following a length step (Huxley & Simmons 1971, Ford, Huxley & Simmons 1977). Velocity and force transients have recently been observed also in smooth muscle (Study III; Hellstrand & Johansson 1979; Mulvany 1979).

The mechanical characteristics of strips of longitudinal smooth muscle from rabbit urinary bladder at different lengths (3:6) and with different modes of activation (3:7, 3:8) have been studied using the isotonic quick release method devised by Jewell & Wilkie (1960) for skeletal muscle. The series elastic element, the force-velocity relation and the velocity transients will be characterized (3:5, 3:7). An analysis of the applicability of the quick release method for the present purpose will be given in the methods chapter.

3:2 Outline of methods

The following refers to the experiments described in sections 3:3, 3:5, 3:6, 3:7 and 3:8. The methods used in 3:4 will be described in that section.

Rabbits weighing between 2 and 3 kg were killed by a blow on the neck. The bladders were dissected out and transferred to oxygenated Krebs solution. Longitudinal strips, containing principally longitudinal muscle (see 2:8), were dissected out from bladders filled with 10 ml Krebs solution. The in-situ length of the preparation at 10 ml will be referred to as L_{10} . This was generally about 5 mm. The preparation weight was from 0.5 to 4 mg. The strip was then mounted in an apparatus that could keep the muscle isometric, and, when desired, release the muscle to previously determined afterloads. 3 types of apparatuses were used. For the determinations of length-tension relations in section 3:3, the apparatus described by Hellstrand, Johansson & Ringberg (1972) was used. The isotonic lever had an equivalent mass about 2.5 g. This made it unsuitable for the study of fast events but it was appropriate for the studies in 3:3. Part of the experiments in 3:3 and all experiments of 3:6 and 3:8 were performed with in an improved apparatus (regarding the design see: Johansson 1973). The equivalent mass of its lever was 150 mg and the compliance of the whole system was 10 $\mu\text{m/g}$. The force and velocity output was recorded on a linear direct-writing oscillograph (Devices MX4). This apparatus gave adequate registrations from about 50 ms after the release. The period 0-50 ms was disturbed by oscillations originating mainly in the force transducer. To enable registration of the events immediately after a release a further improved apparatus was used. Its lever has an equivalent mass of 8 mg (for further details see Sjölin, Hellstrand & Clementz 1978, Hellstrand & Johansson 1979). The output of the force transducer and of the photo-electric device registering lever movement were displayed on a storage oscilloscope (Tectronics 530IN) and recorded by a Grass kymograph camera. Simultaneous slow recordings on a linear direct-writing oscillograph (Devices MX4) were used to monitor the experiments. The signals were also stored on magnetic tape (Tandberg 100 or Thermionic Store 4 instrumentation recorder) for further analysis. This setup was used for sections 3:5 and 3:7.

The preparations were kept in a solution of the following composition in mM: NaCl 120, KCl 6.0, MgCl_2 1.2, CaCl_2 2.5, glucose 11.5 and tris(hydroxymethyl)-aminomethane (Trizma Base, Sigma Chemical Co) 23. (It is referred to as N-tris). The solution had previously been titrated with HCl at 37°C

to pH 7.4. The osmolality, measured by means of an Osmometer 31 LAS (Advanced Instruments Inc), was close to 300 mOsm. The solutions were continuously bubbled with 100 % O₂. The preparations were allowed to accommodate for 1 h before the experiments were started. Two means of stimulation were used. K⁺-contractures were elicited by changing the N-tris solution to a solution (K-tris) in which all NaCl had been exchanged with equimolar amounts of KCl. The muscles could also be stimulated electrically with square waves or AC-current through platinum electrodes.

Passive tension was 2-4 % of maximum active tension unless otherwise stated. Results are given as mean ± SE.

According to Edman & Nilsson (1972) the degree of damping of the lever is critical in experiments on the mechanics of heart muscle due to effects of oscillations on the contractile function. If the lever was underdamped the inertial oscillations were found to deactivate the muscle and the shortening velocity was impaired. Reservations against "the damped-release method" has however been put forward (Gülch 1974). The releases in the present studies are all undamped. To find out whether no deactivating effects of inertial oscillations occur in bladder preparations experiments of the following design were made. Bladder strips were stimulated electrically. Early during the rising phase of one contraction, the muscle was allowed to perform an afterloaded shortening. Later during another contraction, the muscle was released to the previous afterload. If no inactivation occurred during the quick release, the shortening velocity, at a given degree of shortening, should be the same for the afterloaded contraction (devoid of oscillations) and after the quick release (possessing oscillations). Fig 7 gives the results of one experiment. The two shortening curves superimpose well except during the first 75 ms after the quick release. During 0 - 75 ms shortening velocity is actually higher after the quick release. The obvious conclusion must be that inertial oscillations after quick releases have no deactivating effects on the smooth muscle preparation.

3:3 Length-tension relations of bladder strips in vitro

Strips containing longitudinal smooth muscle from rabbit urinary bladder were used (I). After the accommodation period the strips relaxed in Ca²⁺-free N-tris and were then transferred for 5 min to K-tris solution which elicited a contracture, tension reaching a plateau level in 1 - 2 min. The bathing solution was then changed back to Ca²⁺-free N-tris and the strips

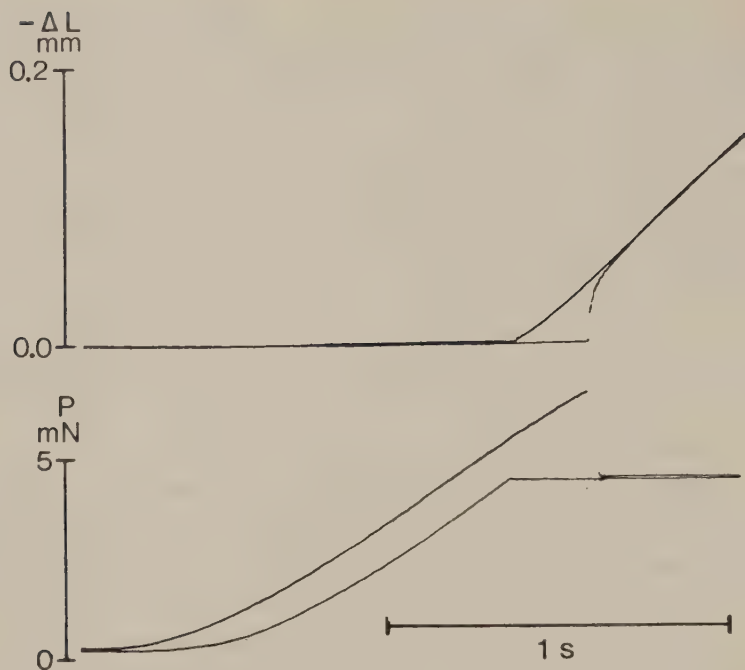


Fig. 7 An afterloaded isotonic contraction and a quick release to the same afterload of an electrically stimulated rabbit bladder strip (length 6.0 mm). In order to superimpose the length registrations the contraction ending with an afterloaded isotonic shortening is delayed in the figure relative to the quick release response. The length change in the quick release registration is typical; an initial elastic recoil followed by an intermediate phase of rapid shortening, and finally a slower shortening that can be superimposed upon the afterloaded shortening. The intermediate phase last about 75 ms.

rapidly relaxed. Time in this solution was 10 min. These steps were then repeated at different muscle lengths. The experiments generally started at L_{10} and the muscles were then shortened in steps. When enough data had been obtained, the muscles were stretched in steps. After the experiments the strips were gently blotted and weighed. Their cross-sectional area at the length where they developed maximum active tension was calculated, assuming a density of 1.0 mg/mm^3 . Fig 8 shows the length-tension relations for 5 strips treated as just described. Active tension (total minus passive tension) starts to develop at a length of about 35 % of L_{10} . Active tension increases with stretch until a maximum is reached at a length of $206 \pm 4 \%$

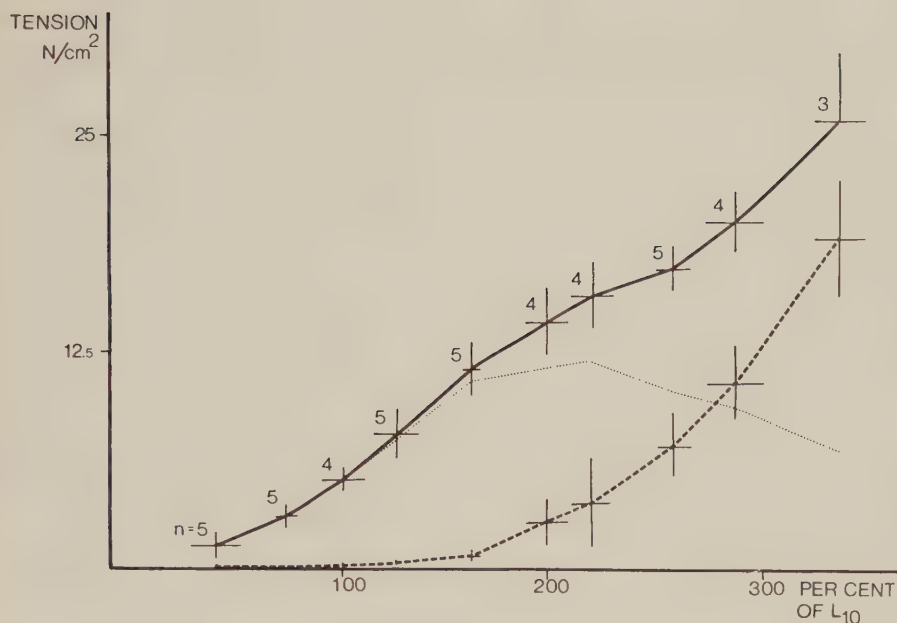


Fig. 8 Mean length-tension relations of 5 strips of longitudinal smooth muscle from rabbit urinary bladder. L_{10} is the length of the strip at a bladder volume of 10 ml. Relative lengths are divided into 10 classes. Continuous line: Total tension. Broken line: Passive tension. Dotted line: Active tension. The bars indicate SD. From I.

of L_{10} ($n = 5$). Maximum active tension was $12.5 \pm 0.4 \text{ N/cm}^2$. Further stretch results in decreasing active and increasing passive tension. After correction for non-muscular tissue (see 2:8) mean active tension is 19 N/cm^2 .

The shape of the length-tension curve obtained here is typical for mammalian smooth muscle (see e.g. Stephens, Kroeger & Mehta 1969, Lowy & Mulvany 1973). The bladder strips begin to develop tension at a length about 17 % of the optimal. This agrees well with the 13 - 25 % obtained by Stephens et al (1969) and differs markedly from skeletal muscle which do not normally develop tension at lengths shorter than 65 % of optimal (Gordon, Huxley & Julian 1966). The absence of Z-discs in mammalian smooth muscle might be of importance for their ability to shorten extensively. It could also be possible that the lesser efficiency of the electromechanical coupling at short muscle lengths reported by Rüdél & Taylor (1971) does not apply to smooth muscle but there is no evidence for this. As shown in chapter 2 no slippage between the muscle cells exist for a bladder radius interval covering the whole rising part of the length-tension curve. Slippage can

therefore be ruled out as a reason for the slow rise in active tension relative to increase in muscle length.

Increasing the Ca^{2+} -concentration in the K-tris solution to 10 mM doubled the contractile force (I) but the shortest length at which active tension occurred was virtually unchanged. Shortening down to 17 % of optimal length thus probably represents the maximal isotonic response. Lowering of extracellular Ca^{2+} to 0.5 mM diminishes isometric force by a factor of ten compared to force output in 10 mM Ca^{2+} . The shortening capacity decreases however, only slightly.

It has been found for skeletal muscle that the active tension developed at a certain length is the same irrespective of whether this length is reached passively or by isotonic shortening from a greater length (Gordon et al 1966). It was observed in the present studies (I) that this is not always the case for bladder strips. A series of 6 experiments was performed to compare isometric and isotonic length-tension relations. The strips were activated by K-tris following the same time schedule as described above, and isometric tension was measured. At every second contraction the muscle was allowed to perform afterloaded isotonic shortenings and its length was varied as above. Up to initial lengths about 165 % of L_{10} the tension produced at a certain length was the same irrespective of how that length was reached. At lengths above 165 % of L_{10} the muscle could no longer shorten isotonicly to the point on the isometric length-tension curve corresponding to the afterload. This "shortening defect" which was most pronounced for intermediate and high afterloads, increased progressively with further increases in length. At very small loads the shortening defect was almost absent. To see if the shortening defect could be due to e.g. overstretch of injured tissue in the knot region three experiments with the following design were performed. Three 0.1 x 0.5 mm silver markers were placed between the muscle bundles in the preparation, thereby dividing it into four segments. Isometric and isotonic contractions were performed and the distances between the markers were measured with a dissection microscope. The inability of the muscles to shorten isotonicly from large starting lengths to corresponding points on the isometric length-tension curve was apparent and this shortening defect occurred in all four segments, i.e. also in the central ones which were unaffected by knot injuries.

Differences between isometric and isotonic length-tension curves resembling those presented above have been noted also by workers using other smooth muscles, e.g. Dobrin (1973) on dog carotid artery, and Stephens & Van Niekerk (1976) on dog trachealis muscle. The phenomenon in these tissues seems to be at least partially reversible and differs in this respect from the irreversible changes described by Peterson & Paul (1974).

No satisfactory explanation for the shortening defect is available. It could be possible that cell slippage exists at extreme lengths but according to section 2:5 the relationship between bladder radius and cell length is linear up to 100 ml volume which corresponds to 215 % of L_{10} and the shortening defect appears already at initial lengths from 165 % of L_{10} . As the tension plateau during a K^+ -contracture lasts for a considerable time, the possibility of a decrease in activation with time as an explanation is unlikely.

The shortening defect is probably of minor physiological importance as it appears only at lengths that correspond to bladder volumes near or above the normal maximum. Ruptures of the mucosa often occur in the rabbit bladder at 100 ml content volume (corresponding to 215 % of L_{10}).

3:4 Calculation of cellular length-active tension relations

The smallest functional component in skeletal muscle is the sarcomere. In smooth muscle it is not known whether a corresponding structural unit exists or not. At present the cell has to be considered the smallest functional part of smooth muscles. Isometric twitches have been elicited in isolated single cells from toad stomach (Fay et al 1976) and shortening velocity was recorded in such preparations by Bagby (1974). To my knowledge no length-tension relations of single isolated cells have been reported.

Whereas single isolated cells produce force only end-to-end, it is possible that cells in situ can transmit force over many parts of their surface to neighbouring cells. In this section we will try to calculate length-active tension relations for a mean guinea pig bladder cell in situ from volume-active pressure relations obtained in vivo. The experiments were carried out on ether-anaesthetized guinea-pigs weighing about 400 g. The abdomen was opened and the bladder was cannulated via the urethra. The ureters were ligated and cut 0.5 - 1 cm from the bladder, and were drawn together with surrounding tissue through plastic tubes with platinum electrodes at each

end. The electrodes were connected to Grass S44 stimulators via a Grass SIU5 isolation unit. The cannula was connected to a Statham P23 DC pressure transducer. Through a branch tube the bladder could be filled with Krebs solution to desired volumes, starting at zero. After the pressure had stabilized the bladder was stimulated for 15 s by a train of supramaximal square waves. Pressure increased rapidly to a maximum and then declined to some extent before the stimulation was ended. The bladder was filled to a new volume and the procedure repeated. When the highest desired volume was reached the bladder was emptied in steps and stimulated again. Time between stimulations was 10 min. Fig 9A summarizes the results. Maximum active pressure was obtained at 0.15 ml bladder volume and pressure in the relaxed bladder was negligible at all volumes. Assuming a spherical form which is

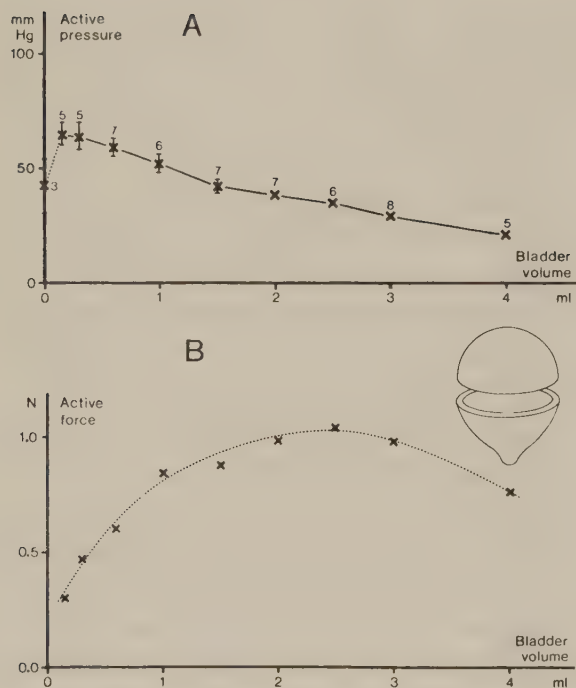
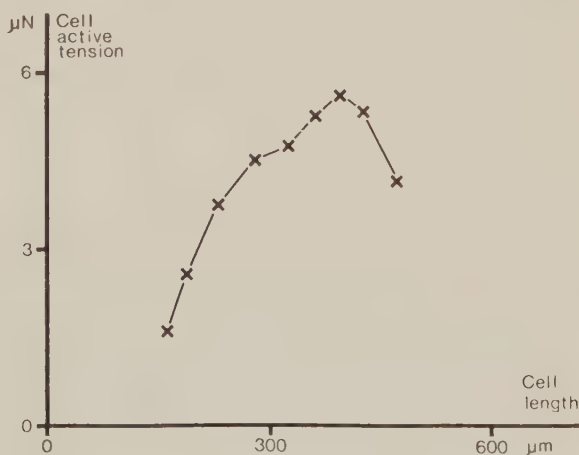


Fig.9 In vivo experiments with bilateral pelvic nerve stimulation. Guinea-pig. A: Active pressure vs content volume. Four animals were used. Mean $\pm$ SE. Numbers of determinations indicated in the figure. Maximum active pressure occurs at 0.15 ml. B: Force produced by the longitudinal muscle holding the two indicated hemispheres together, vs bladder volume. Maximum force at 2.5 ml. From VI.

an appropriate approximation (see above) the volume-pressure relation can be transformed to a volume-force curve. The force trying to separate the two hemispheres in Fig. 9B is $F = r^2 \times \pi \times P$ (r is bladder radius, P is pressure). This separating force is balanced by the force produced by the longitudinal smooth muscle, $F = A \times T$ (A = area of longitudinal muscle, T is tension /unit of area/). The active force developed by the wall of the bladder reached a maximum of 1.04 N at 2.5 ml bladder volume (Fig. 9B).

The total number of longitudinal muscle cells transected according to the bladder model in Fig. 9B was calculated. Mean thickness of the longitudinal layer was measured on the sections used in 2:3. The thickness times the circumference gave the area of longitudinal muscle at the equatorial region. From Fig. 5 the relevant cell packing densities could be obtained. The total number of cross-sectioned longitudinal cells at the equatorial region was 185000 ± 24000 ($n = 6$). Using this figure the force/cell could be calculated at all volumes in Fig. 9. From Fig. 3B the corresponding cell lengths could be obtained. Fig 10 shows the results of these calculations in the form of a cellular length-tension curve. A maximum active force of $5.5 \mu\text{N}$ is developed at a cell length of $400 \mu\text{m}$. This maximal force per cell is comparable to those obtained by Fay (1977) in isolated toad stomach cells, and by Mulvany & Halpern (1976) in vascular smooth muscle. If the length-active tension curve is extrapolated downwards by a straight line the length corresponding to zero tension becomes $110 \mu\text{m}$ which is 28 % of the optimal length. This is higher than the 17 % obtained in 3:3 for whole

Fig. 10 Calculated length-active tension relation for an average individual longitudinal smooth muscle cell of guinea-pig urinary bladder. From VI.



preparations of rabbit bladder strips but this difference may just reflect uncertainties in the calculations.

The number of cells per mm^2 at optimum length is 107000 according to Fig. 5. Active force per cm^2 pure muscle bundle would then be 59 N. This value is high compared to most other vertebrate smooth muscles including those used for the results in section 3:3. One reason is probably that our in vivo experiments are performed on bladders with good metabolic conditions (no impairment of blood flow) and in contrast to strip preparations, there are no injuries caused by knots or dissection procedures. Gabella (1976c) showed that guinea pig taenia coli strips produced more tension with carbachol than with K^+ -high solutions or electrical field stimulation. He obtained $42 \text{ N} \times \text{cm}^{-2}$ with carbachol corresponding to $74 \text{ N} \times \text{cm}^{-2}$ when correction was made for nonmuscular tissue. Stimulation of the pelvic nerves to the bladders could on these grounds be expected to give a more powerful contraction than what is obtained by K-tris in 3:3.

The reason for the high active force in some mammalian smooth muscle is not known. Rosenbluth (1965) showed that the myofilaments of toad jejunum cells are not in parallel with the long axis of the cells. This could be a factor that increases cellular force. However, according to Gabella (1979c) myofilaments in taenia coli cells appear almost parallel to the long axis of the cells at all degrees of shortening. Myosin filaments are $2.2 \mu\text{m}$ long in rabbit portal vein (Ashton, Somlyo & Somlyo 1975) compared to $1.6 \mu\text{m}$ for skeletal muscle (Page & Huxley 1963). According to Rüegg (1971) active force in muscle is directly proportional to thick filament or sarcomere length. Longer thick filaments enable more cross-bridges to work in parallel, thereby increasing force output. Maximum active force for mammalian smooth muscle is about 30 N/cm^2 (Close 1972). If everything else was constant, 41 N/cm^2 ($30 \times 2.2/1.6 \text{ N/cm}^2$) would be expected in skeletal muscle if the thick filaments had the same length as in smooth muscle. The variation in thick filament length can thus not account for the whole difference. The significance of the high actin and low myosin content in smooth compared to skeletal muscle (Murphy et al 1974) remains to be assessed. Smooth muscle has lower tension cost than skeletal muscle (Paul et al 1976, Hellstrand 1977, Johansson 1978) suggesting that the duration of the cross-bridge cycle is longer. If this was due to a longer relative time at the attached state than in skeletal muscle, this would give a higher force output.

3:5 Isotonic behaviour after quick release

In Fig. 7 a typical quick release is shown. The behaviour after the release can be divided into 3 parts. There is an immediate recoil followed by a phase of fast shortening lasting 50 - 75 ms. Finally the shortening slows down and superimposes almost exactly on a corresponding afterloaded isotonic shortening curve. This section will deal with the three phases of the quick release response in the order that they appear.

The immediate recoil has usually been attributed to the series elastic element according to Hill's (1938) two-component model (see e.g. Jewell & Wilkie 1958, Edman & Nilsson 1972, Hellstrand & Johansson 1975). In smooth muscle the recoil of this "series elastic (SE) element" when the muscle is released to zero tension varies considerably. The earlier the study, the more compliant is the SE element found to be [Åberg (1967): 13%, guinea-pig taenia coli; Stephens & Kromer (1971): 7.5%, dog trachealis muscle; Meiss (1978): 4%, rabbit mesotubarium]. This may partly be due to different preparations and methods in the different studies. Fig 11 shows SE

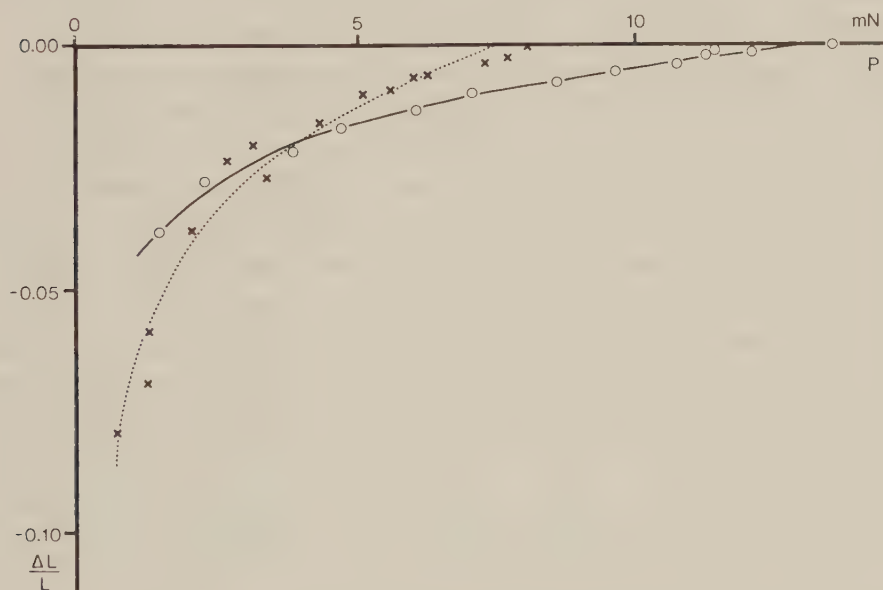


Fig. 11 Series elastic recoil of a rabbit bladder strip and a rat portal vein. Open circles: Bladder (length 4.76 mm, isometric force 3.0 N/cm²). Crosses: Portal vein (length 5.00 mm, isometric force 1.7 N/cm²). Fits to eqn 2 are shown by full and broken lines, respectively. Constant k is 62 for bladder and 36 for portal vein, indicating that the series elastic element is more compliant in the latter. AC-stimulation.

load extension-curves in a rat portal vein and a rabbit bladder preparation. Despite the fact that the same apparatus and the same mode of stimulation were used there is a considerable difference in SE recoil between the two muscles. The experimental data could be fitted to an exponential equation of the following form:

$$P = A \exp(k \times \Delta L / L) + B \quad (\text{eqn 2})$$

The stiffness ($\frac{\Delta P}{\Delta L}$) increases in proportion to force with the proportionality constant k . In paper III k was 91 ± 13 ($n = 7$) for urinary bladder, and 28 ± 5 ($n = 5$) for portal vein. In paper V k was 53 ± 6 , and 34 ± 4 , respectively. The difference in urinary bladder stiffness between the two studies may be related to differences in preparation length and active tension development. It is not known which structures that are responsible for the SE recoil. In skeletal muscle mounted under strictly controlled conditions the recoil is considered to be due to elastic behaviour of the cross-bridges themselves (e.g. Huxley & Simmons 1971). The SE recoil in the bladder muscle is, however, several times larger than in skeletal muscle (regarding the latter see e.g. Bressler & Clinch 1974, Ford, Huxley & Simmons 1977). It is therefore unlikely that a major part of the "series elasticity" would reside in cross-bridges in bladder muscle but it has been suggested that a small fraction may reside in these structures (Hellstrand & Johansson 1979). Meiss (1978) calculated the extension of the SE element for one isolated toad stomach muscle cell in the study by Fay (1977) and obtained a value 2.2% of the cell length. This is only somewhat smaller than the 3-4% maximal SE recoil in bladder muscle found in study III. This comparison could indicate that also in bladder muscle a major portion of the "series elastic element" resides in structures on or in the cell. At present no clearcut explanation for the difference between bladder and portal vein exists. A difference in cell size could play a role but the cell packing density in rat portal vein ($66000 \times \text{mm}^{-2}$, Uvelius, unpublished) is not very different from what is found in bladder (Fig. 5). Another possibility is that extracellular structures are of importance. One difference observed between the two muscle types in the present studies is the richer amount of elastic fibrils in portal vein. This could explain not only the high passive tension in portal vein but perhaps also part of the high compliance of its SE element.

The intermediate phase of the quick release response characterized by fast shortening was first investigated in (III). It has since been described and analyzed by Hellstrand & Johansson (1979) and Hellstrand (1979). It seems possible that it is an effect of cross-bridge rearrangement and change of kinetics after the release. This transient phase subsides after about 75 ms after the release (III).

Determinations of shortening velocity for force-velocity curves were performed at 100 ms after release, thus avoiding effects of the transient. As shown in Fig. 12, the experimentally determined force-velocity points can be fitted to the Hill equation. The curve-fitting was performed either by using a linearized form of Hill's equation (II) or by a computer program that took into account errors in both coordinates (III, IV, V). As will be seen in the following sections $V_{\max}$ varies with muscle length and mode of stimulation.

3:6 Influence of muscle length on force-velocity relations

In this section K^+ -activated rabbit bladder strips were used (see II). $V_{\max}$ was 0.29 ± 0.03 l/s ($n = 8$) at L_{10} . When the muscle was stretched, P_0 (the isometric tension) increased according to the length-active tension relation of the muscle (see 3:3). $V_{\max}$ was also found to be length-dependent even when it was expressed in relation to actual muscle length. At L_{10} a/P_0 was 0.17 ± 0.02 ($n = 8$) and b was 0.052 ± 0.008 muscle lengths per second ($n = 8$). These values are of the same order of magnitude as those found for instance by Mashima & Handa (1969), Stephens et al (1969), and Herlihy & Murphy (1974). As a and b no longer have any certain relation to thermal events in muscle, there is no point in discussing the small differences that exist. Within the interval $0.69 L_{10} - 1.44 L_{10}$ b was constant whereas P_0/a varied in relation to P_0 indicating that also a was length independent over this interval. The results correspond well with those of Gordon & Siegelman (1971) who also found a and b to be constant, although the length interval was smaller in their study.

Hill's equation can be rewritten as $V = [b/(P + a)](P_0 - P)$. With constant values for b and a and with P (the afterload) kept unchanged in the experiment V should vary in proportion to $(P_0 - P)$. Releases to small afterloads were performed in a separate study. The results indicated that there is a linear relationship between P_0 and V at lengths up to about $1.25 L_{10}$. This means that up to $1.25 L_{10}$ the length-active force relation and the

length - $V_{\max}$ curve vary concomitantly with muscle length. At lengths greater than this, shortening velocity increases more than $(P_0 - P)$. The reason for this is not clear. There might be a length-effect on the parameters a and b at these degrees of stretch. It is also possible that the force carried by the contractile units is less than P due to an unloading effect of the passive parallel elasticity which begins to carry force at these lengths.

3:7 Effects of different modes of activation on contraction dynamics

In sections 3:5 and 3:6 it was shown that Hill's equation fits well with observed shortening velocities against different loads. When P_0 was altered by a change in muscle length, $V_{\max}$ was affected to almost the same proportion. This could lead to the assumption that there exists a unique relationship between P_0 and $V_{\max}$. It has been suggested, however, that $V_{\max}$ in smooth muscle may vary with the mode of stimulation, being higher during activation by AC current than during K^+ -contractures (Hardung & Laszt 1966, Hellstrand & Johansson 1975, Murphy 1976). On the other hand P_0 seems also to be higher with AC stimulation (Gabella 1976c). It would be of great interest if it could be shown conclusively that $V_{\max}$ is affected differently than P_0 with different modes of stimulation (AC current, K-tris) since this finding would indicate that the kinetics of the contractile process is different in tonic (K^+) and phasic (AC) activity. The problem has not been studied systematically so far.

In (V) 2-3 min long K^+ -contractures were elicited in rabbit bladder strips. At the peak of each contracture a quick release was made using different afterloads. After a sufficient number of such responses had been recorded, the muscle was kept in N-tris and repeatedly stimulated by AC current (25-100 Hz, $\dot{2} - 4$ V, duration 2 - 5 s) at 1/2 - 1 min interval. Supramaximal AC-stimulation usually gave higher isometric tension than obtained in the K^+ -contractures (cf. Gabella 1976c). Therefore the intensity of the AC-stimulation was adjusted to give the same P_0 as the preceding K^+ -contractures.

The initial (SE) recoils at different afterloads were fitted by a computer program to eqn. 2 (see above). The characteristics of the elastic element were found to be unaffected by mode of stimulation; the constant k of eqn. 2 was 51 ± 6 ($n = 6$) for AC-stimulations, and 54 ± 3 ($n = 6$) for K^+ -contractures. Hellstrand & Johansson (1979) found that the series elasticity was

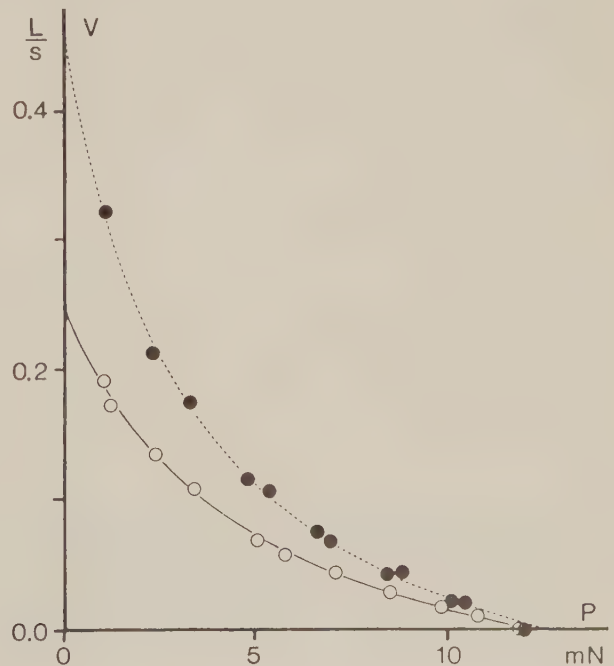
unaffected by changes in temperature of the bathing medium. This together with the apparent lack of influence of stimulation mode (as contrasted to the marked effects on $V_{\max}$, see below) suggests that a major part of the SE recoil is a passive phenomenon.

The length response after the elastic recoil was fitted by a computer program to a double-exponential model (Hellstrand & Johansson 1979) of the form:

$$L(t) = L' + A_1[1 - \exp(-t/\tau_1)] + A_2[1 - \exp(-t/\tau_2)] \quad \text{eqn. 3}$$

The amplitude (A_1) of the fast shortening component was unaffected by mode of stimulation (paper V). The rate constant ($1/\tau_1$) was, however, about 25 % smaller in K^+ -contractures. A comparable effect is seen when bladder muscles are cooled (Hellstrand & Johansson 1979). Their suggestion that the fast shortening component is not a passive phenomenon is further validated by the present results. The fast exponential component subsides rapidly and is practically over at 100 ms after the release, the time at which shortening velocity was measured. The force-velocity values were fitted by a computer program to eqn. 1 in 3:1. $V_{\max}$ was found to be about 30 % lower in K^+ -contractures than in responses to AC stimulation [0.26 ± 0.03 ($n = 6$) l/s vs 0.37 ± 0.05 l/s]. Force-velocity relations from a typical muscle is shown in Fig. 12.

Fig. 12 Force-velocity relations of a bladder strip. Velocities measured 100 ms after the release. Filled circles: AC-stimulation, open circles: K^+ -activation. Fits to Hill's eqn. are shown by broken and full lines, respectively. From V.



There is no straight forward explanation for the fact that $V_{\max}$ is lower in K^+ -contractures than in AC-contractions of equal P_O . It has been reported by Jones et al (1973) that vascular smooth muscle cells swell and that their thick myofilaments disrupt when the vessels are placed in KCl-high media. These authors suggested, however, that the disruption of the thick filament lattice took place during the fixation of the tissue for electron microscopy. They base this suggestion on the fact that the tension output was the same as for K_2SO_4 -solutions which did not cause swelling of the cells. The difference in shortening velocity described above could be an effect of filament disruption if this change occurs also in the living muscle.

Another factor which could contribute to the different $V_{\max}$ values is the time the muscle is activated before the releases are made. The AC-contractions are short-lasting and the releases are performed after 2 - 5 s whereas 1-2 min elapse before a K^+ -contracture reaches its maximum. K^+ -activated slow fibres from *Xenopus laevis* shorten more slowly if a release is made late during a contraction compared with an early release (Lännergren 1978). The finding in paper IV that $V_{\max}$ declines faster than P_O during a single twitch might also be of relevance. Peiper (1979) found that the rate of tension recovery in portal vein after vibration-induced relaxation is slower late during a K^+ -contracture. Altogether these results might indicate that the rate of the bridge-cycling is slowed down with time during prolonged contractions. The findings of Arner & Hellstrand (1980) that, in relation to tension output, the oxygen consumption decreases with time during K^+ -contractures further support this idea. A possible explanation could be that an internal load develops during long-lasting contractions, e.g. due to rigor complexes (Weber & Murray 1973) caused by insufficient ATP supply.

The $V_{\max}$ value for rabbit bladder muscle (0.37 l/s. V) is high compared to many other smooth muscles but much lower than in skeletal muscle (from 7 l/s; Close 1972). The possible relationship between $V_{\max}$ and metabolic rate in smooth muscle is discussed by Hellstrand (1977).

3:8 Single twitches: Shortening velocity and active force

Paper IV gives an account on how P_O and $V_{\max}$ are affected in rabbit bladder strips at 25° C, during single twitches elicited by single supramaximal square wave pulses. P_O and $V_{\max}$ were determined as the intercepts with the

force and velocity axis, respectively, of the computer fits of the data to Hill's equation.

The time from stimulation to peak of the twitch was about 2 s. $V_{\max}$ and P_0 reached their maximum values at the same time, about 1 s after stimulation. $V_{\max}$ then declined rapidly. At the peak of the isometric force, for instance, the $V_{\max}$ value was only 70 % of its maximum. The P_0 value on the other hand, decreased more slowly and is at the peak of the isometric contraction only slightly less than maximum. A comparison of force-velocity curves from the rise and from the peak of the isometric response shows that they differ in the same manner as the curves obtained during AC and K^+ -stimulations respectively in 3:7. The possibility that inhomogeneity of activation could be of importance for the change in the force-velocity relation during the twitch was ruled out. Strips were marked with charcoal grains and were photographed while contracting. Only minor movements of the grains were observed.

The difference in time between the releases during the rise and those at peak of contraction is smaller than the difference between releases performed during AC and K^+ -contractions. They strengthen, however, the suggestion in 3:7 that the duration of activation is of importance for $V_{\max}$ rather than the mode of stimulation.

4. EFFECTS OF PROLONGED BLADDER DISTENSION ON LENGTH-TENSION RELATIONS

In response to an increased functional demand, smooth muscle will hypertrophy (see e.g. Gabella 1975). The development of the hypertrophy is fast as seen e.g. in rat portal vein subjected to increased intramural pressure (Johansson 1976). The cross-sectional area of longitudinal muscle in this vessel has increased considerably within 8-11 days after the pressure rise. This fast hypertrophy shows that there exists an intimate dynamic relation between muscle cell size and the functional demands.

Peterson et al (1974) have induced hypertrophy in rat urinary bladder by injection of paraffine into the lumen. In the present study this method was used on female guinea-pigs weighing 550 to 600 g. Paraffine with a temperature just above melting point (43°C) was injected through a urethral cannula into the bladder of nembutal anaesthetized animals. Each animal had a matched control with the same body weight. Terramycin was added to the drinking water in order to avoid bladder infection. Despite this precaution, half of the paraffine-injected animals had to be discarded due to loss of weight, probably as a result of bladder infection.

After 10 days the animal was killed and the bladder was emptied of urine leaving only the paraffine mass. A longitudinal bladder strip was marked out, measured, dissected free, and mounted in an apparatus that could record isometric tension and isotonic shortening (lever equivalent weight 300 mg; see 3:2). The strip was stimulated by K-tris solution following the same time schedule as in 3:3. The minimum length to which the muscle could shorten actively when unloaded was determined as was the length at which maximum active tension developed. These lengths will be expressed in relation to the in situ length of the strip. After the experiments the strip was blotted and weighed. The bladder was weighed before and after 3 days in 70°C in order to determine the water content of the tissue. The volume of the paraffine mass was measured. The control bladder was filled with Krebs solution to give a content volume equal to the paraffine volume. A strip was then marked out and treated as described above.

Table I gives the results from the 4 hypertrophic bladders and from their controls. The bladder weight increased from 0.43 ± 0.03 g in the controls to 1.30 ± 0.17 g. The control bladders weighed slightly more than guinea-pig bladders in chapter 2 (0.3 g), probably because the animals used here were

larger. There is little inflammatory reaction in the bladders of these selected animals. The increase in bladder weight is therefore not due to inflammatory oedema. The water content did increase, however, from $78 \pm 0.9\%$ in the controls to $86 \pm 0.3\%$ in the hypertrophic bladder. The study did not give any indication regarding structural localization of the increase in water content.

There was no significant difference in optimum length for force generation but this length is difficult to determine due to the broad and flat length-tension curve. The tension output for both controls and hypertrophic bladders in Table I was low compared to those in 3:3 and 3:4. The main reason is probably that the present strips contained all layers (mucosa to peritoneum) and no correction for the amount of non-muscular tissue has been made. The active tension per unit area was considerably lower in hypertrophic bladder (0.91 ± 0.23 vs 1.67 ± 0.15 N/cm²). The same qualitative difference has been described by Johansson (1975) for portal vein

Table I A comparison between hypertrophic (H) bladders and their controls (C). Content volumes between 1.2 and 2.1 ml. The length ($L_{\max}$) at which maximum active tension ($P_{\max}$) is developed is expressed in relation to the in situ length of the strip. Passive and active tension at $L_{\max}$ are expressed in relation to both wet weight/ $L_{\max}$ and dry weight/ $L_{\max}$. The length (L_0) that the unloaded muscle can shorten to is given in relation to the in situ length. The results were analyzed according to student's t-test for paired data. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

	C	H	
bladder wet weight (g)	0.43 ± 0.03	1.30 ± 0.17	**
bladder dry weight (g)	0.09 ± 0.01	0.19 ± 0.02	**
water content (%)	78 ± 0.9	86 ± 0.3	**
$L_{\max}/L_{\text{in situ}}$ (%)	136 ± 15	133 ± 10	
P_{passive} at $L_{\max}$; wet (N/cm ²)	0.62 ± 0.19	0.42 ± 0.17	
P_{passive} at $L_{\max}$; dry (N/cm ²)	2.89 ± 0.95	2.99 ± 1.24	
$P_{\max}$; wet (N/cm ²)	1.67 ± 0.15	0.91 ± 0.23	*
$P_{\max}$; dry (N/cm ²)	7.74 ± 0.85	6.26 ± 1.55	
$L_0/L_{\text{in situ}}$ (%)	60 ± 7	80 ± 8	*

and Gabella (1979b) for intestinal muscle. $P_{\max}$ per cm^2 dry muscle would be $7.7 \pm 0.9 \text{ N/cm}^2$ for the controls and $6.3 \pm 1.6 \text{ N/cm}^2$ for hypertrophic bladders. These values did not differ significantly and might indicate that the decreased tension per unit area seen in hypertrophic bladders is due to water accumulation. Gabella (1979b) observed a considerable increase in intermediate filaments and a relative decrease in myofilaments and he suggested that this might be a factor contributing to the relative reduction in contractile force. He also suggested that the changes in intercellular collagen, that he describes, might be of importance. In the present study passive tension at $L_{\max}$ was higher for control bladders (0.62 ± 0.19 vs $0.42 \pm 0.17 \text{ N/cm}^2$) but if passive tension is considered in relation to dry cross-sectional area the difference disappears. This could indicate that the structures responsible for passive tension are not considerably changed in hypertrophic bladders.

Unloaded hypertrophic strips can shorten less, relative to their in situ lengths, than their matched controls (Table I). This indicates that the relation between the muscle cells or the properties of the contractile proteins within the cells have changed. According to Gabella (1979a) cell length is unchanged in intestinal muscle 3-5 weeks after onset of hypertrophy. Shortening capacity was not determined in that report and the time before sacrifice was longer than in this study. If in spite of this, his results are applicable to the present study they might indicate that the properties of the myofilaments have altered. Comparisons of characteristics of the force-velocity relations between controls and hypertrophic bladders would obviously be of great interest. Such studies have not yet been performed. It is to be noted however, that $V_{\max}$ is lower in papillary muscles from hypertrophic hearts compared to controls (Hamrell & Alpert 1976).

5. GENERAL DISCUSSION

This discussion can be separated into 4 parts: 1. The relation between preparation length (or bladder distension) and muscle cell length, 2. the effect of preparation length on tension development (length-tension relations), 3. the isotonic behaviour of bladder muscle at different lengths and stimulation modes, and 4. effects on length-tension relations induced by bladder hypertrophy.

The cell lengths were measured in papers I and VI using two different methods. The direct relationship seen between preparation length (or bladder radius) and cell length strongly suggests that the cells are coupled in series. This is in accordance with the results from pig carotid artery by Murphy, Driska & Cohen (1977). No slippage between the muscle cells could be detected even at considerable stretch. Evidently results regarding tension output and shortening velocity vs e.g. preparation length represent in a direct way the behaviour of the individual muscle cells.

As there exists a linear relationship between cell length and number of cells per unit cross-sectional area it seems reasonable to assume that cell volume is independent of the degree of passive stretch. Fay & Delise (1973) found that the stereometrically determined volumes of isolated muscle cells from toad stomach decreased up to 30 % when the cells were stimulated and actively shortened. The difference in results could indicate that, whereas passive length changes do not induce net water movement over the cell membrane, active shortening does.

The length-tension relation of bladder muscle has the same general configuration as that of other smooth muscles (for ref. see Murphy 1976). Rabbit bladder strips can shorten to 17 % of the optimal length for active tension development. The slow rise in active tension when a bladder strip is stretched is, as mentioned above, not due to cell slippage. As the cells are coupled in series the same length-tension behaviour is to be expected for individual cells. The study includes an attempt to determine length-active tension relations for single cells in intact guinea-pig bladders (VI). Maximum active tension ($5.5 \mu\text{N}$ per cell) is attained at a cell length of $400 \mu\text{m}$. This active force corresponds to 59 N per cm^2 , a high value compared to 19 N per cm^2 obtained for rabbit bladder strips. It appears unlikely that this difference would be due to the different species used. It is probably so that the intact guinea pig bladder in situ

has a better supply of oxygen and nutrients and that there is not tissue damage of the kind expected to occur when strips are dissected out. These favourable conditions do not apply to the isolated rabbit strips.

The capacity of smooth muscle to shorten extensively in comparison to skeletal muscle could be due to the absence of Z-discs and/or. to better electromechanical coupling at shorter lengths. Variations in extracellular Ca^{2+} greatly influences force output in bladder muscle, but the capacity to shorten is not much changed. Active force per unit cross-sectional area in guinea-pig bladder muscle is higher than in skeletal muscle. Part of this might be explained by longer thick myofilaments as described in other smooth muscles (2.2 μm vs 1.6, Ashton et al 1975). The cell length determinations in the present study (I, VI) show that parallel coupling, which would increase tension output, is virtually absent. The lower tension cost observed for some smooth muscles (Paul et al 1976, Hellstrand 1977) might indicate that in these muscles the crossbridges spend a longer time attached during a cycle than in skeletal muscle.

Hill's muscle model, consisting of a contractile element (CE) in series with a series elastic element (SE) has been applied to smooth muscle by many investigators (e.g. Stephens, Kroeger & Mehta 1969, Gordon & Siegelman 1971, Johansson 1973, Hellstrand & Johansson 1975). The length of the SE and the shortening velocity of the CE depend on the load. According to the classical concept a change in load should instantaneously change SE length and CE shortening velocity. In skeletal muscle this model is an oversimplification as it has been shown that in well controlled studies (e.g. Ford, Huxley & Simmons 1977) a major part of the "series elasticity" is associated with the cross-bridges. Moreover a change in load does not instantaneously change shortening velocity to a new steady level: a transitional phase exists (Civan & Podolsky 1966). Also in smooth muscle research the simple Hill model has been reconsidered. The change in shortening velocity following a load change is not instantaneous (III, Hellstrand & Johansson 1979). An intermediate phase exists characterized by fast shortening. Not until about 75 ms after the quick release is the length record superimposable on an afterloaded isotonic shortening with the same load. The intermediate phase lasts for a longer time than the one described for skeletal muscle. It seems to be associated with cross-bridge events as different modes of activation (K^+ vs AC stimulation) affect V_{max} and the

rate constant of this transient to about the same extent (V). It has also been shown that $V_{\max}$ and the rate constant both decrease when temperature is lowered (Hellstrand & Johansson 1979).

The force-velocity relation for bladder muscle is well approximated by Hill's equation. $V_{\max}$ increases with increasing muscle length to the same extent as isometric force (P_0) in a major part of the rising phase of the length-tension relation. At lengths near those for optimal force, $V_{\max}$ increases more than active P_0 . This might indicate that the dynamic constants in Hill's equation change, but it is equally possible that the force carried by the contractile machinery becomes smaller due to the increasing passive tension at these lengths.

$V_{\max}$ is lower for K^+ -contractures than for AC-stimulations giving the same active force (paper V). The reason for the lower $V_{\max}$ for tonically contracting muscle is not known. In *Xenopus* slow fibres (Lännergren 1978) shortening velocity to a given load decreases with time in K^+ -high media. The reason for the lower $V_{\max}$ in the tonic contractions is not known, but it is possible that the myofilament lattice may be affected in KCl (cf. Jones, Somlyo & Somlyo 1973) or that biochemical changes in the cell (e.g. change in ATP) may influence cross-bridge cycling rate. The SE behaviour is unaffected by mode of stimulation (V) suggesting that its major part is passive. A minor part of the SE in bladder muscle has been suggested to reside in the contractile proteins or structures associated with them (Hellstrand & Johansson 1979, Hellstrand 1979).

Strips from bladders that are hypertrophied due to prolonged distension have the same optimum length for force development as their controls after normalization to their in-situ length. Maximum force is lower in hypertrophied muscle, but if correction is made for the increased amount of water in these preparations, the difference disappears. The hypertrophied muscle cannot shorten to the same extent, when unloaded, as the controls. It is not known if this is due to changes in relations between the cells or to changes within the cells. The latter possibility seems the most probable one as cell length in intestinal muscle does not change when the muscle hypertrophies (Gabella 1979a).

6. SUMMARY

Mechanical characteristics of smooth muscle from rabbit and guinea-pig urinary bladder were examined in relation to bladder morphology. Muscle cell length (determined by nitric acid maceration or morphometry on fixed tissue) was directly proportional to bladder radius (or stretch of isolated bladder strips) indicating a series coupling of the cells. The number of cross-sectioned cells per mm^2 varied between 35000 and 170000 and was found to increase in proportion to bladder radius, indicating that cell volume is not affected by passive length changes. Length-tension (L-T) relations were determined for rabbit bladder strips. Active force was 19 N/cm^2 at optimum length. The strips could shorten actively to 17 % of this length, when unloaded. During the major portion of the rising part of the L-T curve isometric and isotonic L-T relations were identical. At greater lengths the strips failed to shorten to the same length as when contracting from a shorter starting length. L-T relations for a single pig bladder cell were calculated. Max active tension at optimum length ($400 \mu\text{m}$) was $5.5 \mu\text{N/cell}$ corresponding to 59 N/cm^2 pure muscle. Isotonic quick releases were performed on rabbit bladder strips. The immediate series elastic (SE) recoil was followed by a transient phase (75 ms) of rapid shortening, and finally a steady shortening. Max SE recoil was 4 %. SE characteristics were unaffected by mode of stimulation suggesting that it is predominantly passive. V_{max} was 0.37 l/s and 0.26 l/s when strips were activated by AC and K^+ -high media, respectively. The rate constant for the transient was lower for K-activation than for AC, indicating that it is due to events associated with the contractile proteins. Hypertrophic bladder muscle had lower max active tension than controls, but the difference disappeared after correction for higher water content in the former. Hypertrophic bladders could not shorten to the same ultimate length, when unloaded, as their controls.

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